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brainwave-discovery

Bioluminescence-based approach to monitor neural activity in freely moving *Drosophila* larvae

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Background:

Dissecting behavioral circuits requires us to examine activity in the brain as the animal processes sensory information and generates autonomous behavior. With a few exceptions most current methods require a constrained preparation, which does not translate well to application in freely moving animals. We developed a non-invasive bioluminescence-based approach to address this problem; targeting the expression of the calcium-reporter Aequorin (AEQ) to the *Drosophila* larval nervous system to measure neural activity. We report conditions that significantly increase the sensitivity of this assay, allowing us to measure activity in Kenyon cells (KCs) (100s) as well as in smaller populations of neurons, with clear signals obtained from less than 10 neurons in intact animals. Finally, we measure both spontaneous activity and evoked responses from KCs in freely-behaving larvae.

Methods: Bioluminescence principle

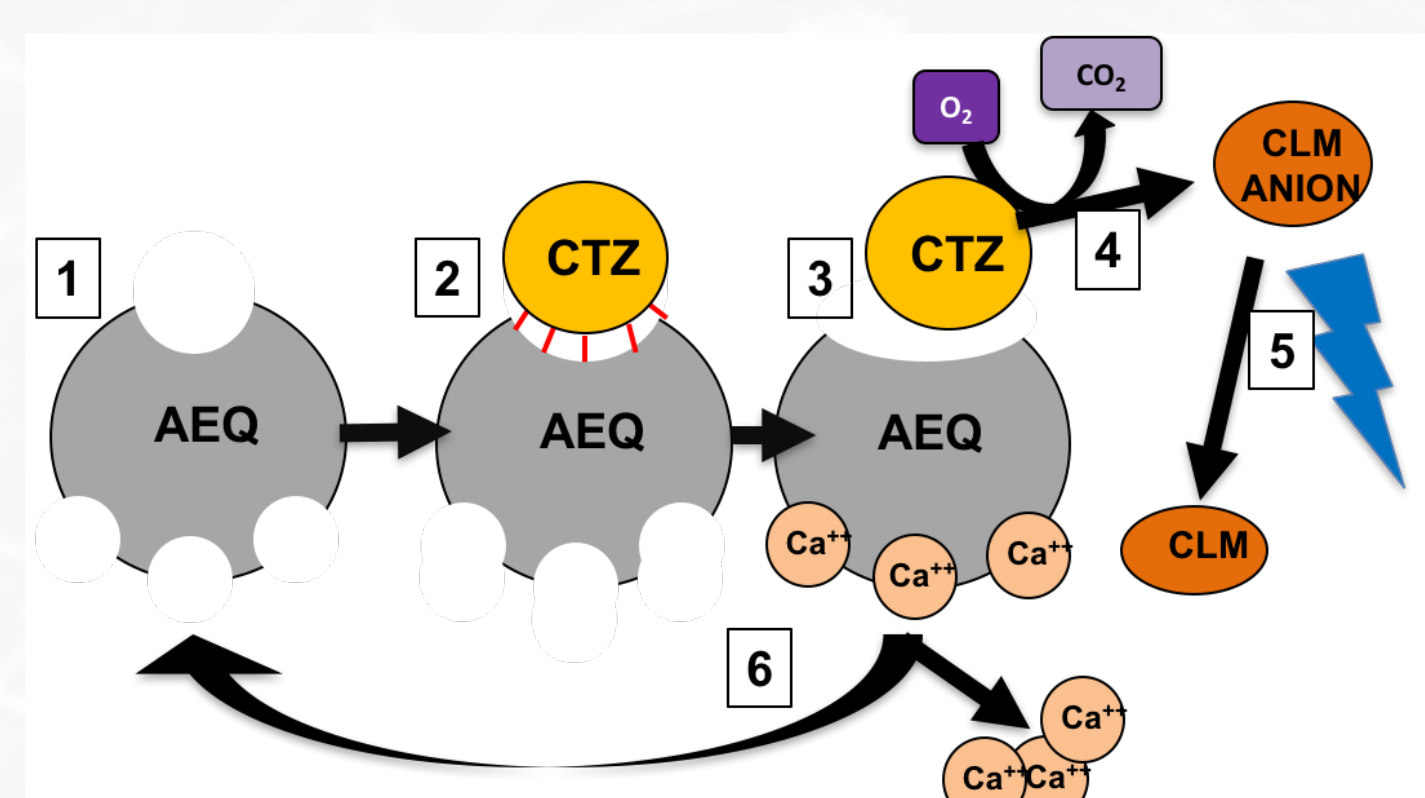


Figure 1. The principle of bioluminescence in *Drosophila* larvae in six steps. AEQ= Aequorin, CTZ= Coelenterazine, CLM= Coelenteramide. In red the covalent bond between CTZ and AEQ, that is broken when Ca^{++} ions bind AEQ. This event leads to a chain of reactions that will produce, eventually, photons within the blue wavelength..

Results (I): Neuroluminescence from intact larvae

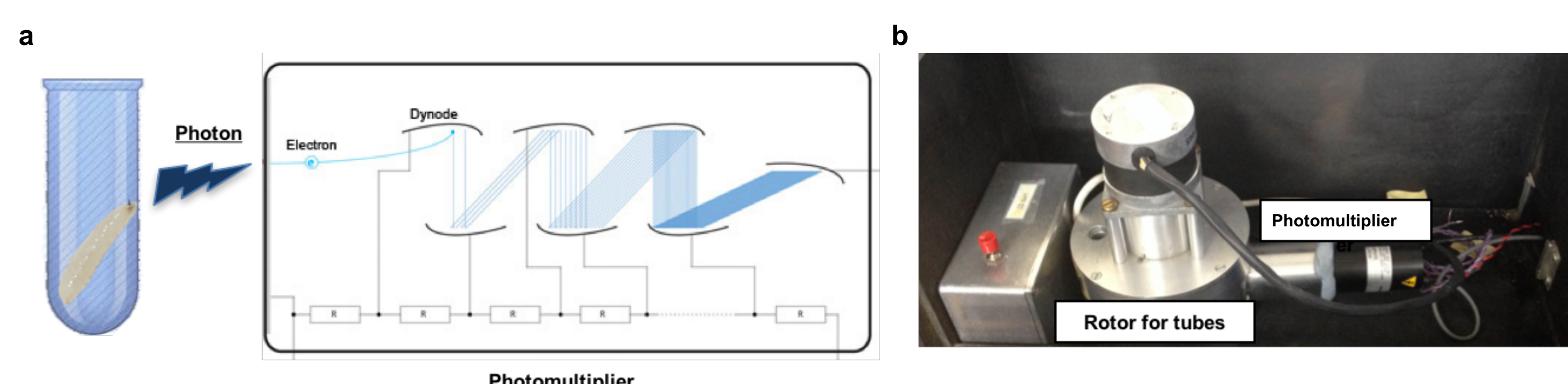


Figure 2. The first set-up used to record bioluminescence from neurons in intact *Drosophila* larvae. a represents the scheme of the analysis, while b the actual machine.

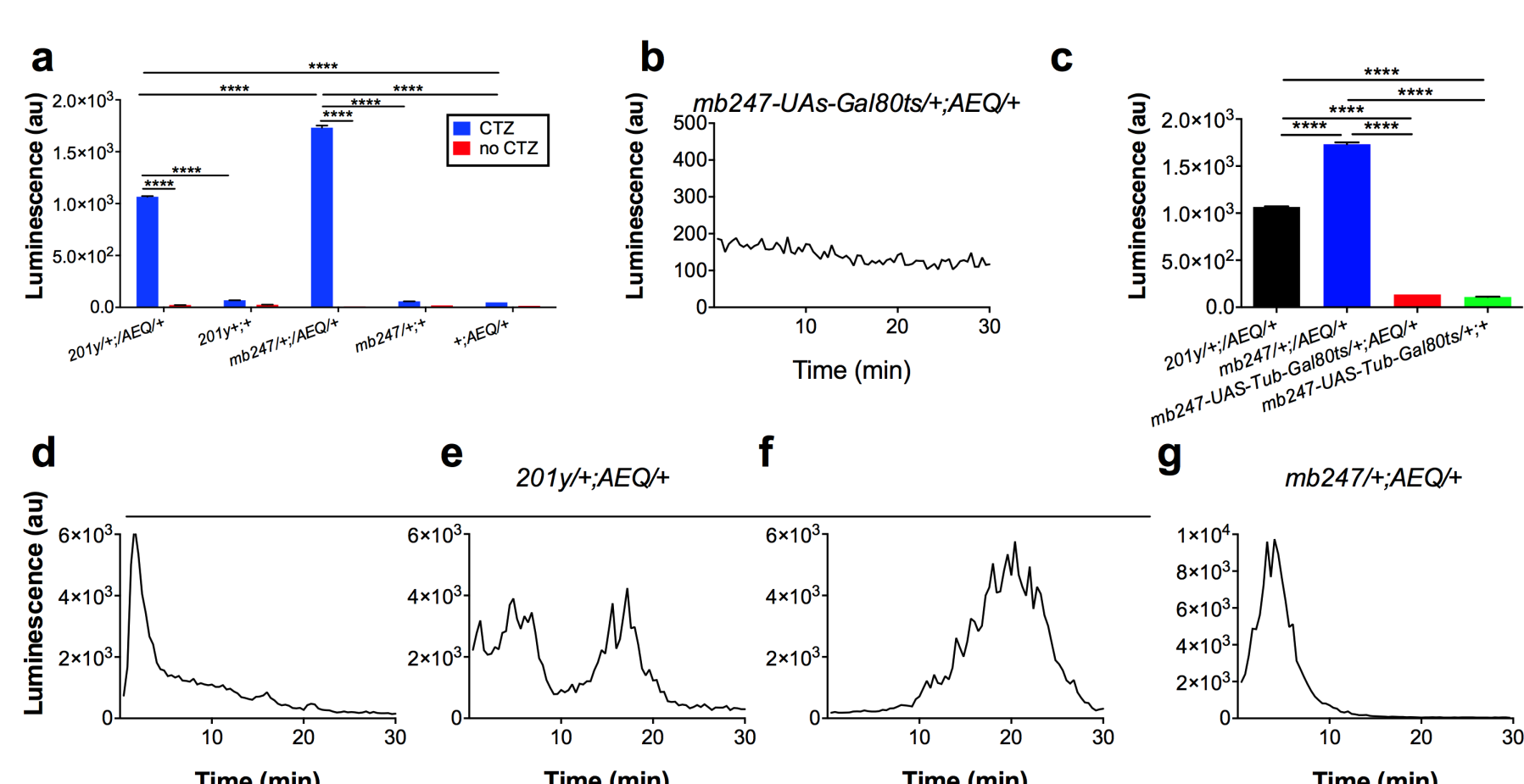


Figure 3. Detection of spontaneous activity of Kenyon cells from intact larvae. a and c represent the mean bioluminescence values, while b, d-g are representative individual examples.

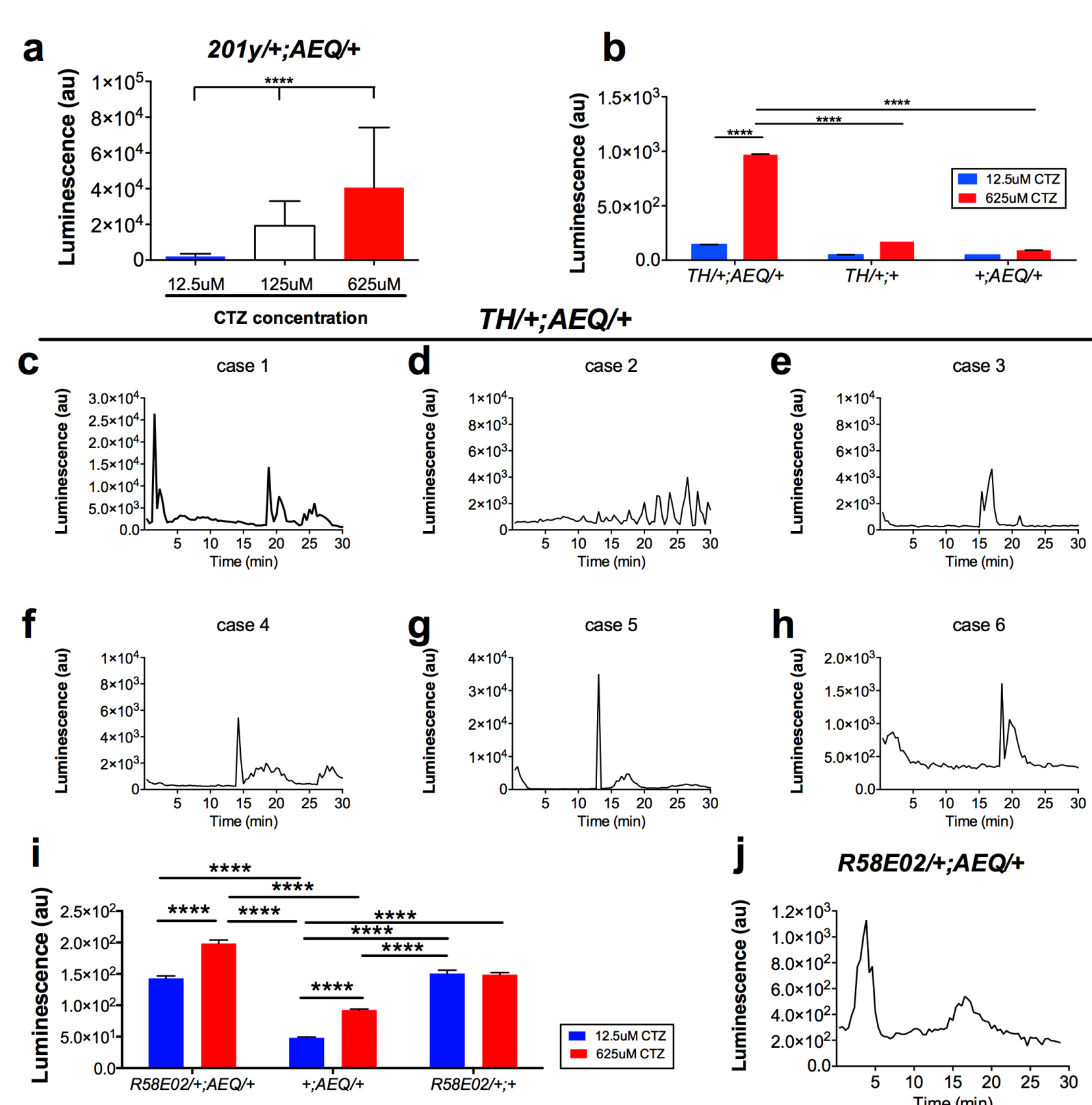


Figure 4. Sensitivity range of the assay from 100s – 6 neurons. a,b and i represent the mean bioluminescence values, while c-h and j are representative individual examples.

Results (II): Neuroluminescence from freely-crawling larvae

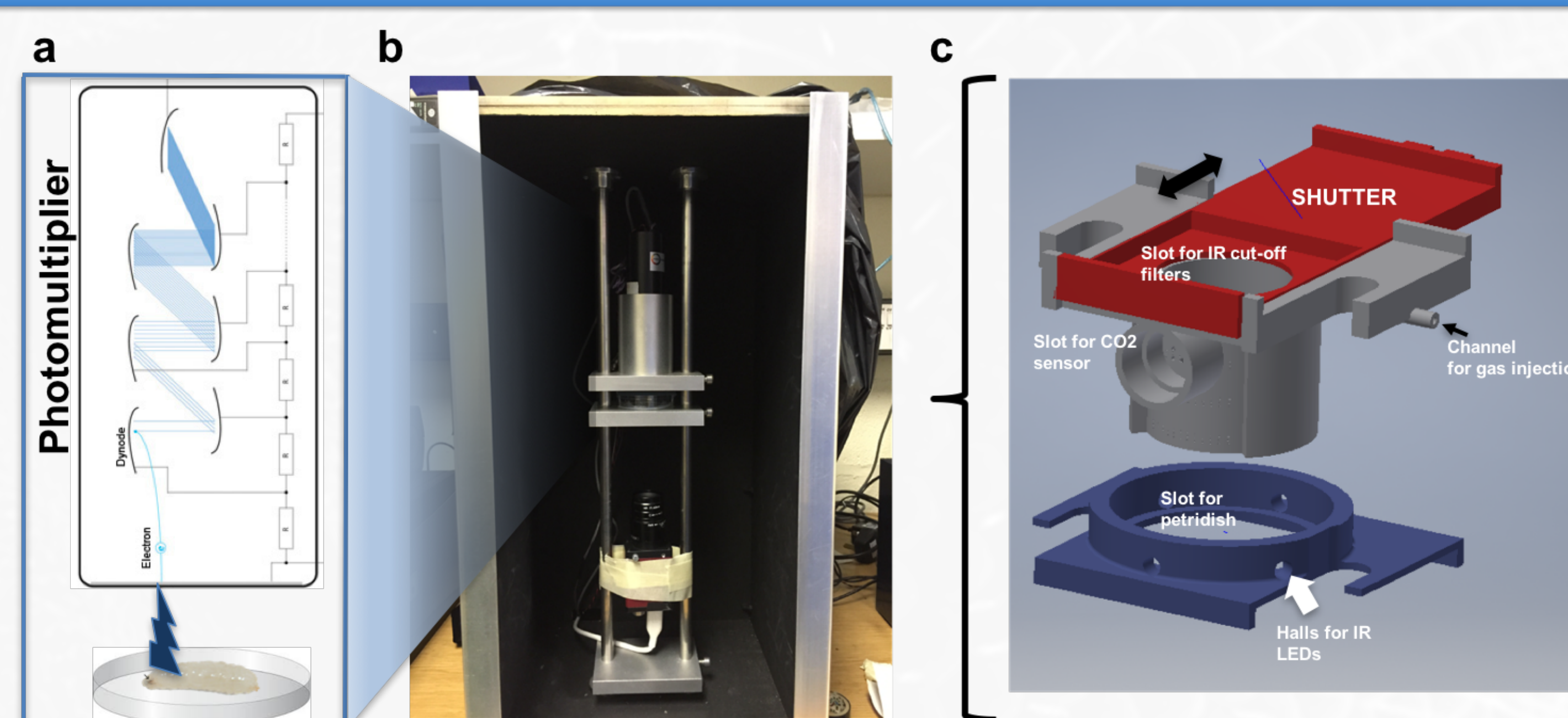


Figure 5. Apparatus designed to record neural activity from intact larvae that are freely behaving. (a) The scheme of the position of the larva with respect to the photomultiplier (PMT). (b) represents the actual prototype of the apparatus. (c) The three-elements set up placed between the arena and the PMT designed to serve a number of scopes: including PMT protection from light and illumination of larva.

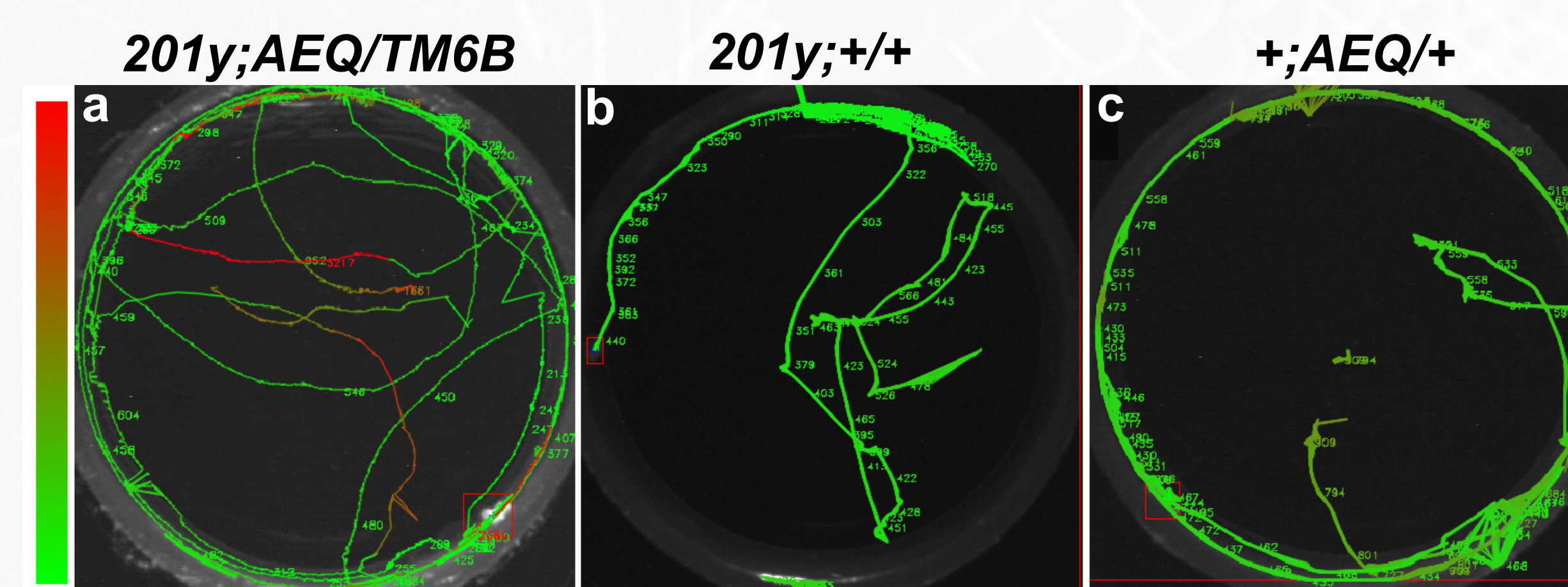


Figure 6. Larval trajectories overlaid with the bioluminescence signal obtained from their KCs in the absence of stimuli. Each plot is 30 minutes long, it contains one animal and the bioluminescence frame-length is 2000ms. Red indicates 2000au (highest signal level).

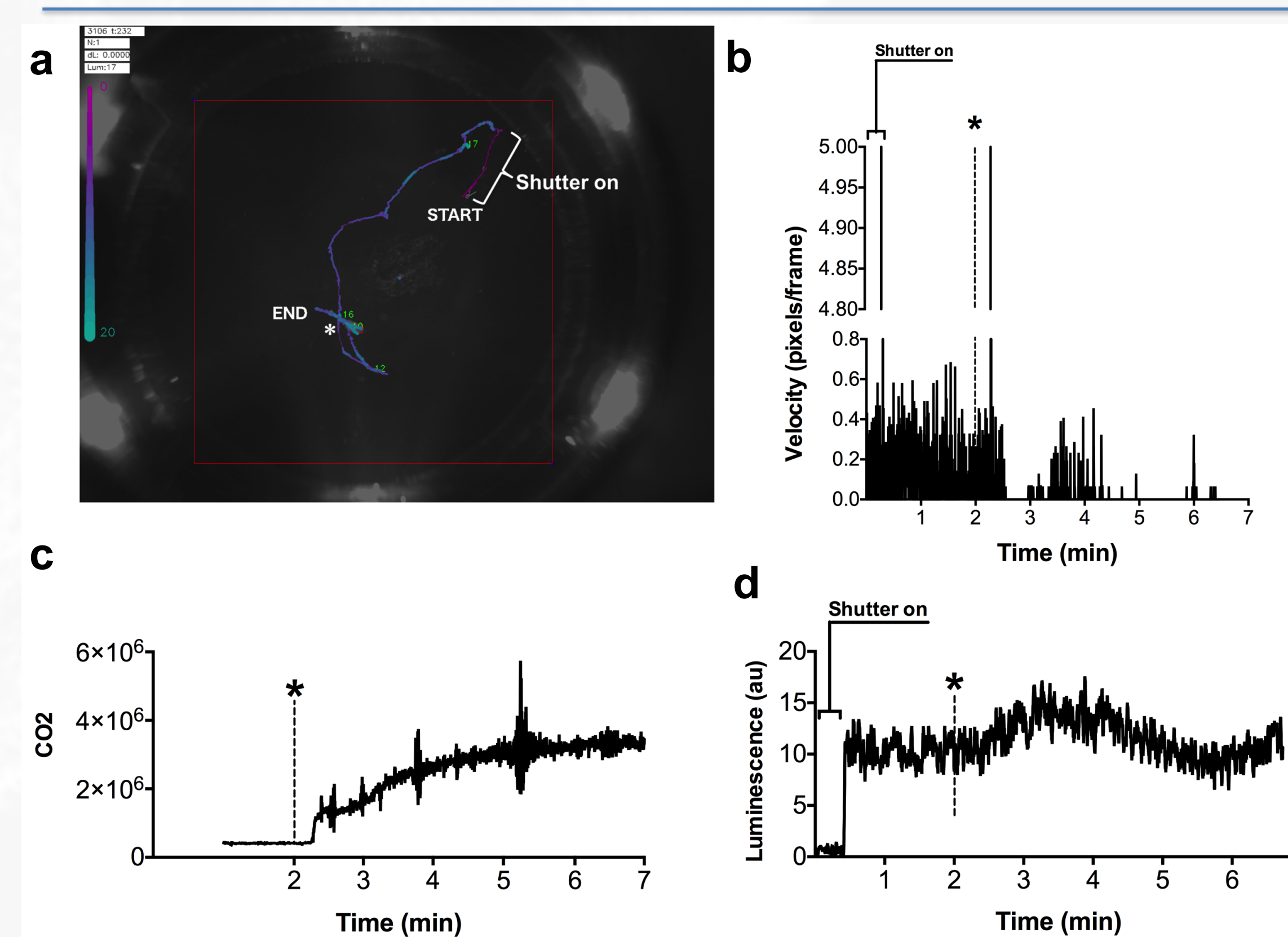


Figure 7. Kenyon cells show increased activity in response to exposure to CO₂. (a) The Trajectory of a single larva colored according to the neuroluminescence (d) emitted from its KCs, where the direction of the track is given by the START and END labels. * indicates the moment the CO₂ stimulus starts. "Shutter on" represents the period of test during which the PMT is protected by the shutter. (b) represents the velocity of the larva, while (c) the CO₂ concentration (ppm).

Conclusions and Ongoing work:

We developed for the first time a method allowing real-time analysis of neuronal activity in intact larvae while they are freely moving.

Our aims for the future are:

- Technical improvement of the system
- Analysis of smaller group of neurons, ideally single neurons
- Study the effect of more inputs on neural activity in the context of behaviors